Differing effects of denosumab and alendronate on cortical and trabecular bone

Roger M. Zebarzea,1, Cesar Libanatib,2, Matthew Austinb,2, Ali Ghasem-Zadeha,1, David A. Hanleyc,3, Jose R. Zanchettad,4, Thierry Thomasc,5, Stephanie Boutroyf,6, Cesar E. Bogadod,4, John P. Bilezikian,7, Ego Seemanab,∗

a Austin Health, University of Melbourne, Melbourne, Australia
b Amgen Inc., Thousand Oaks, CA, USA
c University of Calgary, Calgary, Canada
d Instituto de Investigaciones Metabolicas, Buenos Aires, Argentina
e INSERM U1059, University Hospital of St-Etienne, St-Etienne, France
f INSERM U1033, Université de Lyon, Lyon, France
g College of Physicians and Surgeons, Columbia University, New York, NY, USA

Abstract

Vertebral fractures and trabecular bone loss are hallmarks of osteoporosis. However, 80% of fractures are non-vertebral and 70% of all bone loss is cortical and is produced by intracortical remodeling. The resulting cortical porosity increases bone fragility exponentially. Denosumab, a fully human anti-RANKL antibody, reduces the rate of bone remodeling more than alendronate. The aim of this study was to quantify the effects of denosumab and alendronate on cortical and trabecular bone. Postmenopausal women, mean age 61 years (range 50 to 70), were randomized double blind to placebo (n = 82), alendronate 70 mg weekly (n = 82), or denosumab 60 mg every 6 months (n = 83) for 12 months. Porosity of the compact-appearing cortex (CC), outer and inner cortical transitional zones (OTZ, ITZ), and trabecular bone volume/total volume (BV/TV) of distal radius were quantified in vivo from high-resolution peripheral quantitative computed tomography scans. Denosumab reduced remodeling more rapidly and completely than alendronate, reduced porosity of the three cortical regions at 6 months, more so by 12 months relative to baseline and controls, and 1.5- to 2-fold more so than alendronate. The respective changes at 12 months were [mean (95% CI)]; CC: −1.26% (−1.61, −0.91) versus −0.48% (−0.96, 0.00), p = 0.012; OTZ: −1.97% (−2.37, −1.56) versus −0.81% (−1.45, −0.17), p = 0.003; and ITZ: −1.17% (−1.38, −0.97) versus −0.78% (−1.04, −0.52), p = 0.021. Alendronate reduced porosity of the three cortical regions at 6 months relative to baseline and controls, but further decreased porosity of only the ITZ at 12 months. By 12-months, CC porosity was no different than baseline or controls, OTZ porosity was reduced only relative to baseline, not controls, while ITZ porosity was reduced relative to baseline and 6 months, but not controls. Each treatment increased trabecular BV/TV volume similarly: 0.25% (0.19, 0.30) versus 0.19% (0.13, 0.30), p = 0.208. The greater reduction in cortical porosity by denosumab may be due to greater inhibition of intracortical remodeling. Head to head studies are needed to determine whether differences in porosity result in differing fracture outcomes.

© 2013 The Authors. Published by Elsevier Inc.
**Introduction**

All genetic and environmental factors influencing bone's material and structural strength express their effects through the final common pathway of bone modeling and remodeling [1]. During young adulthood, this cellular machinery maintains bone's material composition and microstructure by replacing a volume of old or damaged bone with an equal volume of new bone; no permanent bone loss or structural deterioration occurs [2,3]. As age advances, and especially after menopause, remodeling becomes unbalanced and accelerated; large numbers of remodeling units, each depositing a smaller volume of bone than they remove, cause structural deterioration [2]. Remodeling upon trabecular surfaces thins and disconnects trabeculae. Remodeling upon endocortical surfaces thins the cortex while intracortical remodeling upon the myriads of Haversian canals enlarges them focally, increasing porosity [3]. Intense intracortical remodeling in the inner third of the cortex adjacent to the medullary canal produces large intracortical pores and cortical fragments that occupy a ‘transitional zone’ between the compact-appearing but increasingly porous cortex and the trabecular compartment.

To inhibit remodeling, antiresorptive agents must either prevent osteoclastogenesis or access existing remodeling sites and reduce resorptive activity or the longevity of osteoclasts already formed and resorbing bone [4]. Antiresorptive efficacy also may depend on the accessibility of treatments to the site being remodeled. Trabeculae are thin plates in close contact with vascular spaces. These plates have a large surface area allowing the adsorption of bisphosphonates into trabecular bone matrix so that bisphosphonate is present in high concentration [5–7]. When osteoclasts excavate and engulf trabecular bone matrix they are likely to also engulf bisphosphonate and inhibit remodeling [4].

Inhibiting intracortical remodeling may be more challenging because cortical bone has less surface area per unit volume of mineralized bone matrix upon which bisphosphonates can be adsorbed. Less bisphosphonate may be incorporated into the large mineralized cortical bone matrix volume resulting in lower bisphosphonate concentrations than in trabecular bone matrix [8]. When osteoclasts tunnel through cortical bone they may be less likely to encounter bisphosphonate within the matrix they engulf so remodeling continues.

Denosumab, a fully human monoclonal antibody, binds to RANKL and prevents its binding with RANK receptors on osteoclasts and osteoclast precursors and so inhibits the synthesis, activity, and lifespan of existing osteoclasts [9–11]. It is not bound to bone and so is widely distributed throughout the skeleton [12]. It inhibits remodeling and reduces porosity to a greater extent than alendronate in non-human primates [13]. In mice, osteoprotegerin (OPG), the endogenous inhibitor of RANKL, reduces porosity and preserves bone strength more than either alendronate or zoledronic acid [14].

Both cortical and trabecular bone determine bone strength: 80% of fractures in women over 65 years are non-vertebral [15], 80% of bone is cortical, and 70% of all appendicular bone loss is cortical and occurs mainly by intracortical remodeling [3]. The resulting increase in intracortical porosity reduces bone strength exponentially [3]. We hypothesized that the greater inhibition of remodeling with denosumab in postmenopausal women will result in a greater reduction in porosity than achieved using alendronate, while effects on trabecular bone will not differ.

**Methods**

The design and primary results of the study are published [11]. This was a 12-month, randomized, double-blind, double-dummy study of 247 postmenopausal women aged 61 ± 5 years with lumbar spine or total hip bone mineral density (BMD) T-score between −2.0 and −3.0 SD assessed using dual-energy X-ray absorptiometry. Treatments were denosumab 60 mg every 6 months, alendronate 70 mg weekly, or placebo. Of the 247 subjects randomized, 146 had results at month 12 as measured by StrAx1.0 software. Missing data was due to movement artifacts or missing serial measurements. The threshold for exclusion of images due to motion artifact is lower than when measuring other parameters such as density. The exclusion of images because of artifacts was done blind to treatment allocation. There were no baseline demographic, biochemical, or densitometric differences between subjects with or without available data and the entire cohort. All subjects received calcium (≥500 mg/day) and vitamin D supplements based on serum 25-hydroxyvitamin D (25(OH)D) at screening. The daily dose was ≥400 IU if 25(OH)D was >20 ng/mL (≥50 nmol/L) or ≥800 IU if 25(OH)D was 12 to 20 ng/mL (30 to 50 nmol/L).

Women were included if high-resolution peripheral computed tomography (HR-pQCT, XtremeCT®) could be performed on at least one wrist. Exclusion criteria were: a fragility fracture after age 50 years, a prevalent moderate to severe vertebral deformity (semi-quantitative criteria); 25(OH)D < 12 ng/ml; conditions affecting bone metabolism; contraindications to alendronate; prior intravenous bisphosphonate; fluoride (except for dental procedures), or strontium ranelate; cumulative oral bisphosphonate ≥3 months; bisphosphonate for ≥1 month within the past year, any use within 3 months of randomization; parathyroid hormone use within 12 months; or other drugs known to affect bone remodeling or density within 3 months of randomization. The institutional review board or ethics committee at each site approved the study. The study was conducted in accordance with all country regulations, the Declaration of Helsinki, and the International Conference on Harmonization Good Clinical Practice Guidelines. All subjects provided written informed consent prior to enrollment.

Ultradiastal radius images were acquired using ScanCo HR-pQCT with an isotropic voxel size of 82 μm [16,17]. Cortical porosity was quantified at baseline and 12 months using StrAx1.0, a software able to automatically quantify the porosity within the compact-appearing cortex and the outer and inner transitional zones of the cortex [18,19]. The outer transitional zone is trabecularized cortex adjacent to the compact-appearing cortex, while the inner transitional zone is trabecularized cortex adjacent to the medullary cavity [19].

StrAx1.0 is available as an online image analysis software (www.straximages.com). The method is accurate in measuring dimensions (total cross-sectional area, areas of compact-appearing cortex, transitional zones, and trabecular compartments) and porosity. The regression between the gold standard micro-CT and StrAx1.0 measurements from HRpQCT has an R² ranging from 0.87 to 0.99. The regression between gold standard scanning electron microscopy (SEM) and StrAx1.0 measurements from HRpQCT images has an R² ranging from 0.91 to 0.99 for areas and porosity. Reproducibility expressed as the root mean square of the coefficient variation (RMS CV%) for areas and porosity measurements ranges from 0.54 to 3.98% [18]. Porosity was quantified as the percent of the total compartment volume occupied by void. Details and validation of the method of quantification of porosity using StrAx1.0 are published [16,18–21]. To avoid overestimating porosity by including under-mineralized bone matrix, quantification of porosity is confined to voxels with attenuation values less than 80% of that produced by fully mineralized bone. Voxels with attenuation values greater than 80% of that produced by fully mineralized bone were excluded from the analysis because pores only produce attenuation below 80% of maximum [19]. Voxels producing attenuation within 80% of maximum contain matrix that has undergone incomplete secondary mineralization (primary mineralization reaches 80% of maximum within a few days of matrix deposition). Thus, there is little, if any confounding effect of mineralization.

Because StrAx1.0 quantifies porosity from all pores including those below the nominal voxel size of the HRpQCT (82 μm), the measurement is sensitive to artifacts such as those introduced by motion. Images with motion artifacts were excluded without knowledge of treatment allocation.

Analyses were performed on all subjects with data with no imputation for missing data and were reported as change from baseline. The
unit of measurement at baseline and endpoint was percent porosity. Density estimates were derived using a kernel density estimator with a Gaussian kernel using Silverman’s approach for selecting bandwidth [22]. Estimates for the changes in porosity and inferential statistics were derived using a random intercept model with subject as the random effect with main effects for treatment, visit, and baseline porosity [23]. The model included interactions between treatment and visit and between baseline porosity and visit. The model allowed for heterogeneity in variance between treatments. Analyses were performed using R version 2.15.0 [24]. The mixed effects models were fit using the nlme package [25].

This study was the first to use porosity as an outcome variable and therefore no power calculations could be done a priori as no preliminary data were available. We conducted a post-hoc evaluation of power from the observed responses. Power ranged from approximately 60% (compact-appearing cortex) to >90% (inner and outer transitional zones and trabecular BV/TV) for the observed alendronate effects and were even larger for the observed denosumab effects. We note however that any statement of post-hoc power needs to be interpreted with caution in the context of a completed study [26].

Results

Baseline characteristics for subjects with evaluable 12-month porosity data are shown in Table 1 and were similar among treatment groups. As shown in Fig. 1, baseline mean and frequency distribution curves of serum CTX did not differ by group. Serum CTX decreased in all groups at 3 months, shifting the distribution of individual values such that serum CTX did not differ by group. Serum CTX decreased in all groups −1.56% (95% CI −1.61, −0.91) versus −0.48% (95% CI −0.96, 0.00), p = 0.012; outer transitional zone: −1.97% (95% CI −2.37, −1.56) versus −0.81% (95% CI −1.45, −0.17), p = 0.003; and inner transitional zone: −1.17% (95% CI −1.38, −0.97) versus −0.78% (95% CI −1.04, −0.52), p = 0.021. Denosumab also produced a more homogeneous response (lower variability) compared with alendronate at the compact-appearing cortex and outer transitional zone.

Alendronate reduced porosity in the compact-appearing cortex, the outer and inner transitional zones relative to baseline and controls at 6 months. Porosity of the compact-appearing cortex and outer transitional zone did not decrease further between 6 and 12 months, but did so only for the inner transitional zone. By 12 months, compact-appearing cortical porosity in the alendronate group was no lower than baseline or controls. Porosity of the outer transitional zone was lower than baseline, not controls, while porosity of the inner transitional zone was lower than at baseline and 6 months but not controls (in whom it decreased).

In multivariate analyses, treatment with denosumab was the strongest predictor of the reduction in cortical porosity, independent of baseline remodeling determined by serum CTX. Improvements in trabecular BV/TV with denosumab and alendronate were significant relative to baseline and controls (both p ≤ 0.001) and did not differ from each other: 0.25% (95% CI 0.19, 0.30) versus 0.19% (95% CI 0.13, 0.30), respectively, p = 0.208 (Fig. 2).

Discussion

We report that (i) denosumab reduced remodeling more rapidly and more completely than alendronate as assessed by serum CTX. By 3 months, women receiving denosumab and controls had almost complete separation of their serum CTX frequency distribution curves whereas the curve for women receiving alendronate overlapped that of controls. (ii) Denosumab reduced porosity at 6 months, further by 12 months, and did so more than alendronate. (iii) Alendronate decreased porosity at 6 months but no further by 12 months in the compact-appearing and outer transitional zones. By 12 months, cortical porosity with alendronate was no different from controls.

These findings confirm and extend the previously reported decrease in remodeling, and cortical porosity in cynomolgus monkeys treated with denosumab [27]. Antiresorptives slow the rate of bone remodeling which in turn slows the worsening of porosity, but does not actually reduce porosity. We propose that the reduction in porosity seen with denosumab is the net result of two processes. At the start of therapy, resorption rapidly ceases in existing cavities and they proceed with their slower refilling phase. As these sites refill, denosumab simultaneously virtually abolishes the birth of new excavation sites producing a net reduction in porosity [27]. Remodeling remains suppressed until remodeling sites reappear shortly before the second injection. With the second injection, resorption at these sites is again stopped, the sites enter their refilling phase while once again, few if any new remodeling sites appear as this second dose again abolishes osteoclastogenesis. Porosity decreases further and is lower than at 6 months, lower than at baseline, lower than in controls, and lower than in the alendronate group.

In alendronate-treated subjects, resorption cavities excavated shortly before treatment also refill but, by contrast, as alendronate only reduces remodeling by 50–60% [28,29], refilling is offset by the appearance of the residual 40–50% remodeling that is not inhibited by alendronate so the net reduction in porosity is less than with denosumab. These residual remodeling sites excavated during the first 6 months enter their refilling phase in the second 6 months but this refilling is now offset by at least an equal number of newly excavated remodeling sites so that there is no further net reduction in porosity between 6 and 12 months. Porosity at 12 months was no lower than at 6 months and was no longer significantly lower than controls given calcium and vitamin D (which also reduced remodeling markers as seen by the shift in the serum CTX frequency distribution curve). Thus, a reduction in porosity by 12 months in the compact-appearing cortex and outer transitional zone was observed with denosumab but not with alendronate. In the inner transitional zone, a greater reduction in

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Age (mm²)</td>
</tr>
<tr>
<td>Trabecular</td>
</tr>
<tr>
<td>Inner transitional zone</td>
</tr>
<tr>
<td>Outer transitional zone</td>
</tr>
<tr>
<td>Compact-appearing cortex</td>
</tr>
<tr>
<td>Percent of total cortical volume occupied by porosity</td>
</tr>
<tr>
<td>Inner transitional zone</td>
</tr>
<tr>
<td>Outer transitional zone</td>
</tr>
<tr>
<td>Compact-appearing cortex</td>
</tr>
<tr>
<td>Distal radius BMD T-score</td>
</tr>
<tr>
<td>Femoral neck BMD T-score</td>
</tr>
<tr>
<td>Total hip BMD T-score</td>
</tr>
<tr>
<td>sCTX, ng/mL, median (IQR)</td>
</tr>
</tbody>
</table>

N = number of subjects with evaluable 12-month porosity data. Data are mean (SD) unless otherwise indicated.
porosity with denosumab than alendronate was observed and porosity in the alendronate group was not different to porosity in controls. In the trabecular compartment, the improvement in BV/TV produced by each drug was similar.

We suggest that this regional specificity may, in part, be a function of the architecture of the bone itself. Remodeling is surface dependent [30,31]. Bisphosphonates adsorb upon a surface and bind to subendosteal mineralized bone matrix. Cortical bone has a low surface area/mineralized bone matrix volume; there is less surface per unit mineralized bone matrix volume for alendronate to be adsorbed upon. Trabecular bone is fashioned as plates with a large surface area/bone matrix volume configuration and trabecularized cortex also has a

Fig. 1. Frequency distribution curves for serum C-telopeptide of cross-linked collagen (CTX) at baseline and 3 months in controls, alendronate, and denosumab-treated women. After 3 months, there was little overlap between denosumab-treated women and controls receiving calcium and vitamin D while there was substantial overlap between alendronate-treated women and controls. The curves at month 3 are truncated at log (0.052 ng/mL), which represents the lower quantifiable limit of the assay.

Fig. 2. The top image shows three-dimensional reconstruction of the distal radius with the compact-appearing cortex (green), outer (white) and inner (red) transitional zone, and trabecular compartment (yellow). Middle images show each of the regions segmented and reconstructed and the graphs show the corresponding changes in porosity and trabecular bone volume fraction at baseline, 6, and 12 months in controls, alendronate, and denosumab-treated subjects. p < 0.05 compared with: abaseline, bmonth 6, ccontrol, and dalendronate.
larger surface area/bone matrix volume configuration than the compact cortex. Concentrations of bisphosphonate are lower in cortical than trabecular bone [8]. Osteoclasts excavating a canal deep within cortical matrix may be less likely to encounter alendronate within matrix allowing them to continue to resorb bone and produce porosity despite treatment (Fig. 3, lower panels). By contrast, denosumab circulates freely to bone surfaces and into remodeling compartments within which it inhibits osteoclastogenesis and so can inhibit remodeling more rapidly and markedly than alendronate in cortical bone, an observation supported by the near complete reduction in bone resorption markers [9,12,27,32,33].

The inner transitional zone is adjacent to the marrow cavity and contains trabecularized cortex and trabeculae. We suggest that alendronate has greater access to remodeling sites in the inner transitional zone than in the compact-appearing cortex. Alendronate is easily adsorbed upon trabecular surfaces and distributes within the relatively smaller mineralized bone matrix volume so that actively-resorbing osteoclasts are likely to encounter and engulf bone matrix containing alendronate and so be inhibited upon trabecular surfaces [4,34]. Both drugs allow refilling of excavated trenches upon trabecularized cortex and trabeculae with similar reductions in new remodeling sites.

To explain these observations, we speculate that the 50 to 60% reduction in serum CTX with alendronate represents the net result of a near complete reduction in remodeling of trabecular bone but much less of an effect upon the deeper cortical surfaces. This would explain the lesser effect of alendronate on cortical porosity but similar benefits of alendronate and denosumab in trabecular bone (Fig. 3, upper panels). It also explains the lack of improvement in cortical vBMD at the distal radius using alendronate [9–11], but the increase in distal radius BMD consistently observed with denosumab [35–37].

Preclinical studies support these observations. In a mouse model with high cortical remodeling, OPG, the endogenous inhibitor of RANKL, reduced porosity and improved bone strength whereas larger doses of alendronate and zoledronic acid than used clinically had lesser effects on porosity and strength. This cannot be explained by differences in drug dosages as the benefits of OPG and the bisphosphonates were similar at trabecular sites [14]. Similarly, OPG reduced cortical porosity more greatly than zoledronic acid in a rat model of adjuvant arthritis, and denosumab reduced cortical porosity more than alendronate in nonhuman primates [13,38].

Further distinctions between the treatments may be relevant. The earlier and more complete inhibition of remodeling by denosumab is also likely to be the result of rapid and full inhibition of the activity and life span of osteoclasts in remodeling sites existing at the time of treatment [39]. This would produce a more shallow resorption cavity which may then be more completely refilled by the ensuing bone formation, reducing structural decay [34]. Bisphosphonates do not prevent osteoclastogenesis. To inhibit remodeling, bisphosphonates must first be adsorbed upon the endosteal surface and bind to matrix which is then engulfed by osteoclasts, following which, resorptive activity is inhibited. Thus, some erosion must occur before bisphosphonates can stop resorption.

If these observations are correct, they are of potential clinical significance. While vertebral fractures and trabecular bone loss are hallmarks of osteoporosis [1,40,41], non-vertebral fractures account for 80% of all fractures [15]. Cortical bone is remodeled more slowly than trabecular bone, but across life, cortical bone loss is 2 to 3 times greater than trabecular bone loss in absolute terms because the skeleton is 80% cortical; only 20% is trabecular [3]. About 70% of all appendicular bone loss is cortical and occurs by intracortical remodeling which increases porosity, an important cause of susceptibility to non-vertebral fractures. A small increase in porosity disproportionately reduces resistance to bending; this is more so in cortical than trabecular bone [42,43].

Non-vertebral anti-fracture reduction is 20 to 30%, less than half the vertebral fracture risk reduction reported in most trials [44].
explanation may be the differing access of drugs to intracortical remodeling sites initiated upon Haversian canals within the large cortical matrix volume [4,34]. Risedronate has a lower mineral binding affinity than alendronate and penetrates deeper into cortical bone [4,34]. Risedronate reduced non-vertebral fracture rates in two of the three main trials [45–47], while alendronate did not report [48,49]. Nakamura et al. reported that in a 24-month study of 1194 postmenopausal Japanese women and men (placebo, n = 480; denosumab 60 mg every 6 months, n = 472; or open-label alendronate 35 mg weekly, n = 242) [50], new or worsening vertebral fractures occurred in 8.5%, 2.4%, and 5.0% of women, respectively (p = 0.0001 denosumab versus placebo). Major non-vertebral fractures occurred in 3.9%, 1.7%, and 2.3% of women, respectively (p = 0.057, denosumab versus placebo). Thus, numerically, fewer fractures occurred in the denosumab than alendronate group but statistical analyses comparing the two antiresorptives was not reported. Moreover, women treated with denosumab in the pivotal phase 3 trial, although a placebo comparator arm was not available in the 4th and 5th years, had a low reported non-vertebral fracture rate, an observation not reported for alendronate or zolodronic acid, the latter also having high affinity for bone mineral [51].

This study has limitations. StrAx1.0 analysis does not quantify pore size and number so that the relative contribution of reductions in pore number versus pore size to the reduction in porosity cannot be determined at this time. Measures of porosity using StrAx1.0 are more sensitive than measures of density to motion artifacts and this resulted in loss of some images.

In summary, this is the first randomized double-blind, placebo controlled trial comparing the effect of two remodeling suppressing therapies on intracortical porosity in vivo. Denosumab reduced remodeling more rapidly, more completely and decreased porosity more than alendronate. Given the exponential relationship between porosity and bone stiffness, partly reversing cortical porosity is likely to contribute to reductions in fracture risk. Whether this greater reduction in porosity translates into better anti-fracture efficacy will require additional comparator trials.

Acknowledgments

Amen Inc. sponsored this study. We are thankful to Michelle N Bradley, PhD for providing formatting and editing support on behalf of Amgen Inc. and Heather Hartley-Thorne for providing graphic support with funding from Amgen Inc. Author JPB received support from NIH grant DK 32333. All authors participated in the design or implementation of the study, and/or the analysis or interpretation of the findings, and had access to the study data. All authors contributed to the development and critical revision of the manuscript and approved the final version for submission. Author MA accepts primary responsibility for the integrity of the data analysis.

References
